Vomeronasal Function

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Abstract

This contribution briefly explores some unanswered questions about vomeronasal organ function, and introduces other contributions from the symposium 'Vomeronasal Function', presented at the XVIII Meeting of the Association for Chemoreception Sciences (1996). Key publications appearing since the symposium are also listed.

This symposium from the Association for Chemoreception Sciences XVIII meeting brings together new information on vomeronasal function, beginning with that topic's sudden blossoming in late 1995. Reports include Catherine Dulac's and Richard Axel's discovery of genes coding for putative vomeronasal receptor proteins; Changping Jia's and Mimi Halpern's discovery of complementary zones of expression of different G-proteins in the vomeronasal epithelium and in different regions of the accessory olfactory bulb (AOB); and the first recordings of responses to putative pheromones from isolated mammalian vomeronasal receptor cells by Robert Moss. As a balance to these studies focused on the mechanisms of peripheral vomeronasal sensitivity, Barry Keverne summarizes work on the Bruce effect, from his and other laboratories, that together constitutes the most complete account of a vomeronasally mediated whole-organism response. The introduction gives a background to the organization of the vomeronasal system, and introduces several questions about vomeronasal function that remain unanswered by the new findings—or by even more recent findings—some of which are also briefly summarized.

Is the vomeronasal organ a pheromone detector?

Defining pheromones as 'chemicals released by one member of a species and detected by another to their mutual benefit', there is clear evidence for pheromone communication in mammals (the focus here). There is clear evolutionary pressure to develop an effective communication system by mating pheromones that facilitate successful reproduction either by causing a physiological adjustment of the reproductive state (a priming pheromone effect) or by eliciting mating behavior (a signaling pheromone effect). Hamsters, mice and garter snakes, among other species, use mating pheromones that act through the vomeronasal organ (VNO). Snakes also clearly use their vomeronasal organs for

other functions, including prey trailing and prey attack, that would not meet the criterion for pheromone communication indicated above. We do not know whether mammals also use their VNOs for non-pheromonal functions, but that is not an impossibility. We also know that the VNO can respond to off-the-shelf chemicals such as amyl acetate, albeit at relatively high (low micromolar) concentrations. So, simply because a substance stimulates the VNO does not mean that it must be a pheromone. Conversely, not every- thing that acts as a pheromone need stimulate the VNO. Induction of a receptive posture in sows (Dorries et al., 1997) and nipple finding by newborn rabbits (Hudson and Distel, 1986) both appear to depend on the main olfactory system. Furthermore, the facilitation of mating in hamsters, which definitely depends on chemosensory cues, can be accomplished by main olfactory input in experienced males with vomeronasal lesions—that is, in males that had mating experience before the lesion (Fernandez-Fewell and Meredith, 1994; Pfeiffer and Johnston, 1994).

VNO anatomy: why is the sensory epithelium sequestered?

In rodents, the paired VNOs lie one each side of the base of the nasal septum, anterior and ventral to the main olfactory epithelium. Each is a blind-ending tube open at the anterior end. The sensory epithelium is thus sequestered away from the main nasal cavity. Stimuli probably reach the vomeronasal duct opening by dissolving in mucus at the nasal vestibule, i.e. at the nostril. For non-volatile substances, this presumably would follow nasal contact with the stimulus. The openings of large nasal glands, including the lateral nasal glands, open in the vestibule and their secretions contribute to the mucus stream that flows down the septum and posteriorly along the floor of the nasal cavity past the VNO duct. These secretions contain proteins, including 'odorant binding protein' and 'vomeromodulin'

(Krishna *et al.*, 1994), that can bind small molecules. In many other mammals, the VNO opens into the naso-palatine, or incisive, canal, which connects the floor of the nasal cavity with the roof of the mouth. This arrangement allows stimuli to enter from the mouth, perhaps via licking, and perhaps assisted by the facial movements of Flehmen (Melese d'Hospital and Hart, 1985). In either arrangement, there are generally large blood vessels adjacent to the VNO lumen that can act as a 'vomeronasal pump' to suck in mucus and any dissolved substances (Meredith and O'Connell, 1979). This mechanism for relatively rapid delivery of substances to the sensory epithelium would not be necessary if that epithelium were freely exposed in the nasal cavity like the main olfactory epithelium. Thus, we should ask why is it necessary to sequester the vomeronasal epithelium. It seems unlikely that it would be to avoid inappropriate activation of behavior by stimulation at the wrong time or by the wrong substances, because the pump seems to be activated in response to any novel situation, i.e. is not really very selective (Meredith, 1994). Two other possible reasons for sequestration are that (i) the receptor system is more vulnerable than the olfactory receptor system to damage by exposure to continuous nasal airflow; and (ii) there is something special about the secretions from the vomeronasal glands that is important for receptor function. Moss *et al.* (1996) have reported an enhanced response from isolated vomeronasal receptor cells in the presence of nasal mucus, but the mechanism for this effect is as yet unclear.

What is the vomeronasal transduction mechanism?

Within vomeronasal receptor neurons, the transduction mechanism differs from that of the main olfactory receptors. The putative receptor protein genes of the main olfactory system, first reported by Buck and Axel (1991), are rarely if ever expressed in vomeronasal neurons. Instead, a separate family of genes coding for a different family of seven-transmembrane-domain receptor-like proteins was shown to be expressed in vomeronasal receptor neurons by Dulac and Axel, as presented in this symposium. Very recently, this family has been supplemented by an additional family also expressed in vomeronasal but not olfactory receptor neurons, and reported nearly simultaneously by three laboratories (Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997). Furthermore, the characteristic components of the main olfactory system are not found in vomeronasal receptor neurons (Liman, 1996). The stimulatory $G_{\alpha s}$-protein, G_{olf}, the adenylate cyclase type III and the α-subunit of the cyclic-nucleotide-gated (CNG) channels are all absent. Two other G-proteins were shown to be differentially distributed in opossum vomeronasal epithelium, defining two classes of receptor neurons, as presented by Mimi Halpern in this symposium.

Neurons with somata located superficially in the epithelium express $G_{i\alpha 2}$, while those with somata deep in the epithelium express G_o. This distribution is now confirmed in the mouse and rat by Jia and Halpern (1996), and by work from Buck's, Dulac's and Ryba's laboratories. These three laboratories have also shown that the pattern of expression for the G-proteins are closely but not exactly matched by the patterns of expression of the two families of putative vomeronasal receptor genes (Herrada and Dulac, 1997; Matsunami and Buck, 1997; Ryba and Tirindelli, 1997). The further steps of possible transduction cascades linked to these putative receptors and the G-proteins are not yet known. In non-mammalian vertebrates, and recently in mammalian preparations, there is accumulating evidence for an IP_3-mediated second messenger system (Luo *et al.*, 1994; Taniguchi *et al.*, 1995; Inamura *et al.*, 1997; Wakesa and Anholt, 1997) as well as a cyclic nucleotide response (Luo *et al.*, 1994; Taniguchi *et al.*, 1996, 1997). The absence of the α-subunit of the CNG channels, which confers their cyclic nucleotide sensitivity, means that changes in cyclic nucleotide levels may not have a direct action on membrane conductance. The expression of the (non-cyclic-nucleotide-gated) β subunit of the CNG channel in vomeronasal neurons (Liman, 1996) and its ability to form nitric oxide gated channels (Broillet and Firestein, 1997) are intriguing, but its contribution to transduction is uncertain in that significant levels of nitric oxide synthase enzyme are not found in the receptor epithelium (Matsuda *et al.*, 1996). Other novel contributors to potential transduction mechanisms include modulation of the electrogenic sodium–potassium pump, shown to hold frog vomeronasal receptor neurons unexpectedly hyperpolarized (Trotier and Doving, 1996).

Receptor neuron response to putative pheromones

The first report of electrical responses to potential pheromone chemicals in isolated mammalian vomeronasal receptor neurons was by Robert Moss. As presented at this symposium, Moss proposed that the pheromone response could be hyperpolarizing, involving a decrease of an ongoing inward current [and associated with a decrease in cAMP levels (Zhou and Moss, 1997)]. A recent abstract from another laboratory also suggests a decrease in spike rate as a response to urine extracts in vomeronasal receptor neurons from mice (Yu *et al.*, 1997). Recordings from turtle vomeronasal nerve bundles show a clear increase of activity with stimulation (Tucker, 1971) by 'off the shelf' chemicals (not pheromones). These data show a greater response of vomeronasal nerves to lower members of a homologous series of fatty acids and a greater response of olfactory nerves to higher members. This presumably reflects the preferential partition of the smaller, more water-soluble compounds in the mucus of the anterior nasal cavity, where

the exposed vomeronasal epithelium is located in turtles. A similar preference for molecules that are more water soluble (perhaps by virtue of attachment to binding proteins) for delivery to the sequestered, mucus-lined vomeronasal organ in mammals might also be expected.

Afferent coding and analysis

Whatever response is generated in vomeronasal receptor neurons, it must be carried to the AOB by patterns of action potentials in vomeronasal afferent axons. Theoretically, a decrease in firing rate carries equal information to an increase in firing rate, as shown by vestibular input during horizontal angular acceleration of the head and, less clearly, by the 'on' and 'off' retinal ganglion cell pathways. However, a relatively high spontaneous activity is required to provide a reasonable dynamic range for signaling different intensities by decreases of firing rate. We lack reliable evidence for the unstimulated firing rate of vomeronasal neurons *in vivo*. *In vivo* recordings in the AOB of halothane-anesthetized hamsters suggest a low firing rate for most neurons, which would limit the response range, although suppression of ongoing activity was observed to some stimuli (Meredith, 1982, 1991 and unpublished data). Suppression may be due to lateral inhibition from excited AOB neurons via granule cell reciprocal synapses (Rheinhardt *et al.*, 1983; Kaba and Nakanishi, 1995). Excitation of the AOB by vomeronasal stimulation appears to be the norm in rodents (Rheinhardt *et al.*, 1983; Dudley and Moss, 1995; M. Meredith, unpublished data).

A surprising corollary of Mimi Halpern's discovery of two segregated populations of G-proteins in the vomero-nasal epithelium is the separate projection of the neurons containing these G-proteins to different (anterior and posterior) regions of the AOB. This division of the AOB into G_o-related and G_i-related zones reflects earlier findings of differences in lectin and monoclonal antibody binding. Jia and Halpern have recently (1997) shown that AOB mitral cells are also segregated such that each receives input from glomeruli in only one zone (although with some overlap in secondary dendrites), suggesting a differential analysis of signals from the two classes of neurons, and possibly the transmission of information to different central targets. There are as yet no reports of differential projections of the anterior and posterior AOB, nor of differential activation during VNO stimulation with chemical stimuli.

Vomeronasal system function in behaving animals: AOB memory

Barry Keverne summarizes here an extensive series of studies by his own and other laboratories, tracing the pathways and processes controlling the Bruce effect. The blockage of pregnancy by chemical stimuli from males of one strain, in female mice impregnated by males of another strain, has long been known to be dependent on

vomeronasal input, and probably to involve dopaminergic inhibition of prolactin release from the pituitary. Implantation failure does not occur on exposure to the impregnating (stud) male because the female somehow recognizes and ignores chemical signals from the stud. A series of experiments suggested that the neural changes underlying recognition (i.e. learning) occurred in the AOB (Kaba and Nakanishi, 1995). The proposed mechanism involves a general norepinephrine-induced decrease in feedback inhibition during mating, presumably by a decrease in GABA release from granule cells, allowing an overactivation of those mitral cells that respond to the stud's pheromones. The overactivation leads in turn to strengthening of those mitral cell's synapses with granule cells. Subsequently, in the absence of NE, these synaptic changes produce a selective increase in feedback inhibition and, thus, a desensitization of AOB mitral cells that were responding to stud pheromones. The location of the recognition/memory process to the AOB suggests that the VNO mediates individual recognition. It seems unlikely that different strains of mice use different pheromone substances, suggesting that discrimination between strains might be due to strain differences in major urinary proteins (MUPs). These are unlikely to be pheromones in their own right, but a combination of small-molecule pheromones and strain-identifying MUPs could give the requisite degree of selectivity in response. MUPs are known to bind small molecules and could act as carriers to deliver them to the VNO. This hypothesis implies that the vomeronasal receptors would respond differentially to MUPs, perhaps when bound to small molecules—bringing us once again back to the unanswered questions about the delivery of stimuli and the transduction mechanisms of the vomero-nasal receptor system.

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